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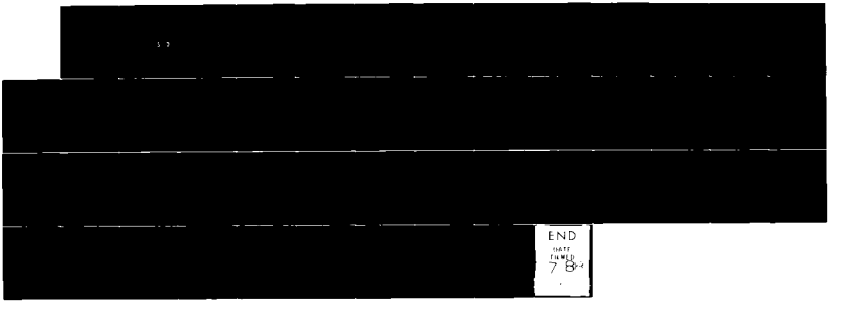

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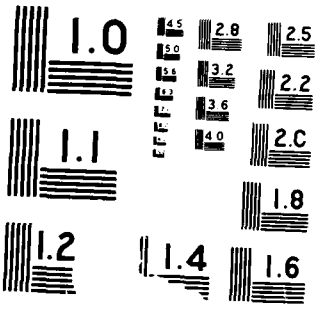
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DRUG EVALUATION IN THE PLASMODIUM FALCIPARUM-AOTUS MODEL (U)

ANNUAL REPORT

Richard N. Rossan

(For the period August 1, 1985 to July 31, 1986)

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Infections of two strains of Plasmodium falciparum, Uganda Palo Alto (chloroquine sensitive) or Vietnam Smith (chloroquine resistant), in Aotus trivirgatus, were used to evaluate the blood schizonticidal and curative activity of experimental antimalarial drugs. WR 250547 and WR 250548, stereoisomers of floxacrine, administered in combination each at doses of 0.5 or 1.0 mg base per kg (x 3 days) cleared and/or cured Vietnam Smith infections. Other doses ratios were equally effective indicating a potentiating effect of WR 250547 for WR 250548. Three 9-phenanthrenemethanols were evaluated against Vietnam Smith infections: neither the chloride or biquinate salts of halofantrine were effective, at the doses used; WR 122455 cured these treatment failures as well as primary infections. A single intravenous dose of 2-fluoro-l-histidine of 50.0 or 100.0 mg base per kg had no effect upon Uganda Palo Alto strain parasitemias. When 2-iodo-histidine was administered, either as a single intravenous dose of 200.0 or 400.0 mg base per kg or as three oral doses of 100.0 mg base per kg, no antimalarial activity was observed. Sodium artesunate,

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18. acridinol
    2-iodo-histidine
    sodim artesunate

19. administered intravenously at a dose of 30.0 mg per kg (x 2 days), temporarily suppressed Uganda Palo Alto parasitemia. Trials are in progress to adapt the UNC-W2-MEF clone of the CDC Indochina III strain of P. falciparum to Aotus.

SUMMARY

The primary goal of these studies was to evaluate experimental antimalarial drugs in a non-human primate model: blood-induced infections of Plasmodium falciparum in the Panamanian owl monkey Aotus. Two strains of falciparum malaria, Uganda Palo Alto (sensitive to chloroquine and quinine, resistant to pyrimethamine) and Vietnam Smith (resistant to chloroquine, quinine and pyrimethamine) were used.

Previous evaluation of two stereoisomers of floxacrine indicated that WR 250547 cured Vietnam Smith infections when administered at doses of 1.0, 4.0 or 16.0 mg base per kg (x 3 days). WR 250548, however, did not clear and/or cure Vietnam Smith infections when administered at similar doses. When both drugs were administered, in combination, against Vietnam Smith infections a potentiating effect was observed. WR 250547 and WR 250548, each administered at a dose of 0.5 or 1.0 mg base per kg (x 3 days) cleared and/or cured Vietnam Smith infections. Other dose ratios of 0.5 and 2.0, 2.0 and 0.5, 1.0 and 4.0, and 4.0 and 1.0, were equally effective in curing infections of the Vietnam Smith strain.

Three 9-phenathrenemethanols were evaluated against infections of the multidrug resistant Vietnam Smith strain. The chloride salt of halofantrine, WR 171 669AM, cured two infections out of a total of 22 treatments. Doses of 2.9, 5.8 or 11.7 mg base per kg (x 3 days) were not effective against primary parasitemias. The biquinate salt of halofantrine, WR 171699AP, did not clear primary parasitemias when administered at dose of 2.9, 5.8 or 11.7 mg base per kg (x 3 days) and after retreatments cured one of 21 infections. WR 122455 at a dose of 5.8 or 11.7 mg base per kg (x 3 days) cured 3 of 4 primary infections, and all recrudescences following treatment with either WR 171669AM or WR 171699AP.

A prior evaluation of 2-fluoro-l-histidine (WR 251835) showed that a dose of 25.0 mg base per kg (x 7 days) suppressed parasitemia of the Uganda Palo Alto strain and a 50.0 mg base per kg dose was toxic by the sixth day of administration. A re-evaluation of 2-fluoro-l-histidine administered as a single intravenous dose of 50.0 or 100.0 mg base per kg had no effect upon Uganda Palo Alto parasitemias. The 2-iodo-histidine analogue was administered as a single intravenous dose of 200.0 or 400.0 mg base per kg. No activity against infections of the Uganda Palo Alto strain was observed. The monkeys were re-treated orally with three 100.0 mg base per kg doses, but this regimen had no effect upon the parasites.

A pilot evaluation of sodium artesunate, a derivative of Qinghaosu, showed that intravenous administration of a 30.0 mg per kg (x 2 days) dose suppressed temporarily Uganda Palo Alto parasitemia in one Aotus.

Trials are in progress in adapting the UNC-W2-MEF clone of the CDC Indochina III strain of P. falciparum to Aotus. To date, parasitemias greater than 1,000 per mm^3 have been obtained only in a splenectomized Aotus of Colombian origin.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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EXPERIMENTAL PROCEDURES

Two monkey-adapted Plasmodium falciparum strains, Vietnam Smith (resistant to maximally tolerated doses of chloroquine, pyrimethamine, and quinine), and Uganda Palo Alto (sensitive to chloroquine and quinine, resistant to pyrimethamine) were used to induce experimental malaria infections in Aotus trivirgatus for the evaluation of the antimalarial efficacy of candidate drugs. Infected blood, with sodium citrate (2.5%) as the anticoagulant, from untreated Aotus was diluted appropriately with chilled saline (0.85%), such that each milliliter contained 5,000,000 parasites, and this amount was injected into the saphenous vein of experimental and control monkeys.

Blood films, prepared and examined daily beginning on the first post-inoculation day, were stained with Giemsa. Parasitemias were evaluated as follows: negative, if no parasites were detected on a thick blood film after examination for at least 5 minutes; <10 parasites per cmm, if positive only on the thick blood film; parasite enumeration was by the Earle-Perez method and reported as the number of parasites per cmm.

Blood films from untreated Aotus, serving as passage and/or control subjects, were prepared and examined daily during the primary patent period, and daily thereafter for at least three consecutive days after parasites could last be detected on thick blood films. When parasitemia had cleared, films were made and examined twice weekly until a total of 100 negative days had been recorded. If a recrudescence occurred, blood films were obtained again on a daily basis.

The schema depicted in Figure 1 represents the design of a typical drug evaluation study. Parasitemias were evaluated daily (or twice daily) during the treatment period and until blood films were negative for at least seven consecutive days. The frequency of smearing was then reduced to two times per week (Monday and Thursdays or Tuesdays and Fridays). If no recrudescences occurred during a 100 day examination period, the infection was considered to have been cured.

Drug doses were calculated as mg base per kg of body weight. Stock solution of water soluble compounds, at appropriate concentrations, were prepared with distilled water and stored at 8°C for the treatment period. If a compound was water insoluble, a suspension of the requisite amount of drug was prepared daily with 0.3% methylcellulose (in distilled water).

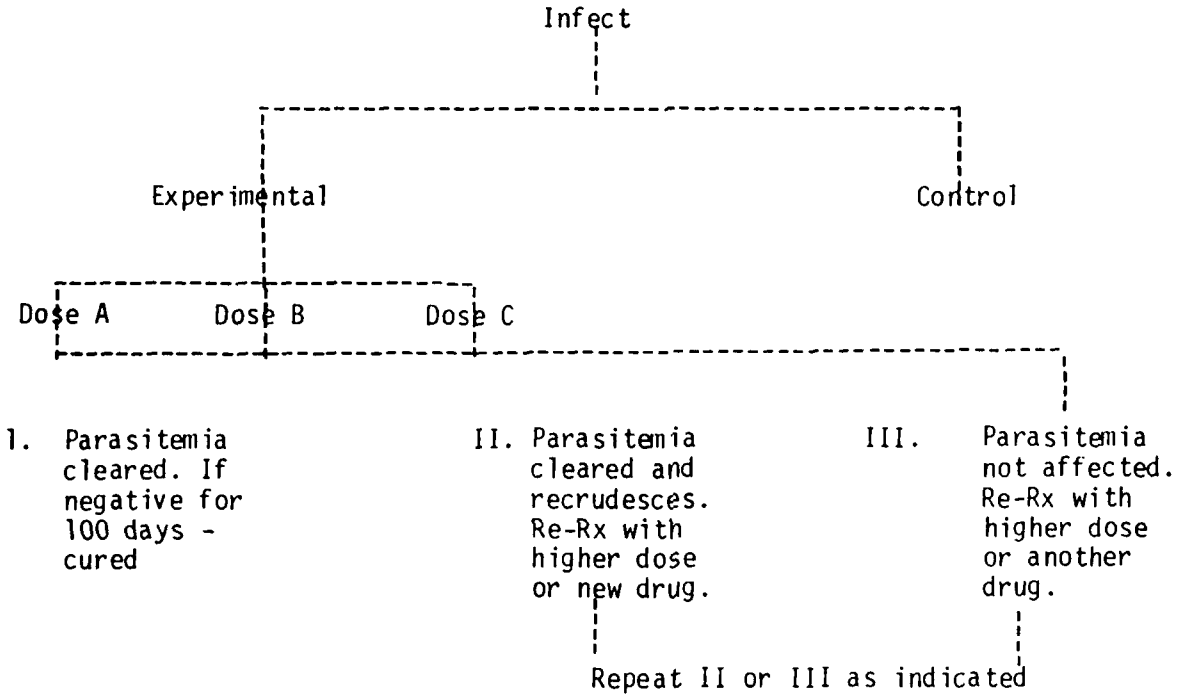
Oral administration of drugs was effected by gastric intubation with a 14 French catheter. The total amount of fluid administered, drug solution or suspension, and rinse was 14 ml. As will be indicated in subsequent sections, some drugs were administered other than by gastric intubation.

FIGURE 1

SCHEMA FOR DRUG EVALUATION AGAINST

PLASMODIUM FALCIPARUM

INDUCED INFECTIONS IN AOTUS TRIVIRGATUS



ASSESSMENT OF THE ANTIMALARIAL ACTIVITY OF WR 250547AA (BN: BK 51630) AND WR 250548AA (BN: BK 51621) IN COMBINATION, AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Both of these acridinol compounds are stereoisomers of floxacrine. The published (1) antimalarial activity of floxacrine against the multi-resistant Vietnam Smith strain of P. falciparum indicated that the total-course CD_{50} and CD_{90} for previously untreated infections were 56.0 and 154.0 mg per kg, respectively. These doses were 6 x and 17 x greater than the 8.75 mg per kg course dose necessary for regular clearance of parasitemia.

Results of the evaluation of the two stereoisomers, singly, against infections of the Vietnam Smith strain were presented in a previous Annual Report (2). The data showed that WR 250547 at a dose of 1.0 mg base per kg (x 3) cured 1 of 2 primary infections and that doses of 4.0 or 16.0 mg base per kg (x 3) cured all primary infections. Primary Vietnam Smith infections were not cleared by WR 250548 at doses of 1.0 or 4.0 mg base per kg (x 3 days); a dose of 16.0 mg base per kg (x 3 days) cleared parasites, but did not cure the infection. Retreatment with higher doses was not uniformly curative.

Subsequently, evaluation of WR 250547 in combination with WR 250548 showed that a potentiating effect was obtained in vitro against P. falciparum and in vivo against P. berghei. (3). These observations provided the impetus to re-evaluate the combination of the two stereoisomers against Vietnam Smith infections in Aotus. The results of this study are presented in Tables 1,2 and 3. Equal doses, 0.5 mg base per kg (x 3 days), of WR 250547 and WR 250548 cleared the parasitemia, but did not cure the infection. Re-treatment with the drugs each at a dose of 1.0 mg base per kg (x 3 days) cured the infection, and this dose of each drug cured a primary Vietnam Smith infection.

Administration (Aotus 11980) of WR 250547 at a dose of 0.5 mg base per kg (x 3 days) in combination with WR 250548 at a dose of 2.0 mg base per kg (x 3 days) only cleared the parasitemia. Retreatment with WR 250547 (1.0 mg base per kg x 3 days) plus WR 250548 (4.0 mg base per kg x 3 days) suppressed parasitemia. A second retreatment with WR 250547 at a dose of 2.0 mg base per kg (x 3 days) plus WR 250548 at a dose of 4.0 mg base per kg (x 3 days) cleared parasitemia, but did not cure the infection. Additional treatment was not possible due to insufficient drug.

A dose of 1.0 mg base per kg (x 3 days) of WR 250547 administered in combination with WR 250548 at a dose of 4.0 mg base per kg (x 3 days) cured the infection in Aotus 11856. The infection in Aotus 11977 was cured with WR 250547 (2.0 mg base per kg x 3 days) plus WR 250548 (0.5 mg base per kg x 3 days). WR 250 547 at a dose of 4.0 mg base per kg (x 3 days) plus WR 250548 at a dose of 1.0 mg base per kg (x 3 days) cured the infection in Aotus 11908.

CONCLUSION

The potentiating effect of WR 250547 for WR 250548 observed initially in the P. berghei rodent model has been confirmed in the P. falcipaum - Aotus model. The dosage combination evaluated of the two drugs show significantly greater activity of WR 250548 when administered with WR 250547. The one failure in Aotus 11980 to cure the infection may have resulted from the induction of parasite resistance to the drugs or the inability of the monkey to absorb/metabolize the drug (s).

TABLE 1

DETAILED ACTIVITY OF THE COMBINATION OF WR 250547AA (BK 51630) AND WR 250548AA (BK 51621) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
11803	0.5a 0.5b	0.9	18	15	19	2	1	<0.01	0	0	0	0
11976	1.0a 1.0b	1	3	5	0.5	0.6	0.2	0.3	0	0	0	0
11803r	1.0a 1.0b	17	11	4	3	<0.01	<0.01	<0.01	0	0	0	0
11980	0.5a 2.0b	0.8	3	3	0.5	0.1	<0.01	0	0	<0.01*	0	0
11856	1.0a 4.0b	1	10	3	2	0.9	<0.01	0	0	0	0	0
11980r	1.0a 4.0b	<0.01	<0.01	1	0.4	0.2	<0.01	<0.01	<0.01	0.1	0.2	0.8
11977	2.0a 0.5b	2	5	6	0.7	1	0.2	<0.01	0	0	0	0
11908	4.0a 1.0b	2	15	9	1	1	0.8	0.4	<0.01	0	0	0
11980rr	2.0a 4.0b	2	1	3	0.1	0	0	0	0	0	0	0

a= WR 250547AA
b= WR 250548AA
*= drug form

TABLE 2

SUMMARY OF THE ACTIVITY OF THE COMBINATION OF WR 250547AA (BK 51630) AND WR 250548AA (BK 51621) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Px to Parasite Clearance	Days from Final Rx To Recrudescence	Notes
		None	Suppressed	Cleared			
11803	0.5a 0.5b			+	7	18	Re-Rx, higher dose
11976	1.0a 1.0b			+	7	n.a.	Cured
11803r	1.0a 1.0b			+	7	n.a.	Cured
11980	0.5a 2.0b			+	9	14	Re-Rx, higher dose
11856	1.0a 4.0b			+	6	n.a.	Cured
11980r	1.0a 4.0b		+		n.a.	n.a.	Re-Rx, higher dose
11977	2.0a 0.5b			+	7	n.a.	Cured
11908	4.0a 1.0b			+	8	n.a.	Cured
11980rr	2.0a 4.0b			+	4	19	Drug Q.N.S. for Re-Rx

a = WR 250547AA
b = WR 250548AA

TABLE 3

SUMMARY OF THE ANTIMALARIAL ACTIVITY OF THE COMBINATION OF WR 250547AA (BK 51630) AND WR 250548AA (BK 51621) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

MALARIA	DOSE mg/kg		PRIMARY TREATMENTS		REPEAT TREATMENTS		TOTAL TREATMENTS	
STRAIN	TOTAL	DAILY	CLEARED	CURED	CLEARED	CURED	CLEARED	CURED
Smith	1.5a 1.5b	0.5 0.5	1/1	0/1			1/1	0/1
	3.0a 3.0b	1.0 1.0	1/1	1/1	1/1	1/1	2/2	2/2
	1.5a 6.0b	0.5 2.0	1/1	0/1			1/1	0/1
	3.0a 12.0b	1.0 4.0	1/1	1/1	1/1	0/1	2/2	1/2
	6.0a 1.5a	2.0 0.5	1/1	1/1			1/1	1/1
	12.0a 3.0b	4.0 1.0	1/1	1/1			1/1	1/1
	6.0a 12.0b	2.0 4.0	1/1	0/1			1/1	0/1

a= WR 250547AA
b= WR 250548AA

ASSESSMENT OF THE ANTIMALARIAL ACTIVITIES THREE 9-PHENANTHRENEMETHANOLS AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

The antimalarial activities in owl monkeys of diverse 9-phenanthrenemethanols have been reported (4). Two drugs in this class, WR 171669 (subsequently called halofantrine) and WR 122755 were highly active. WR 122455 was four times as active as chloroquine against chloroquine - sensitive P. falciparum strains and WR 171669 was equal to the activity of chloroquine.

Evaluation of these two agents in human volunteers (5) infected with the multi-drug resistant Vietnam Smith strain of P. falciparum showed that WR 122455 at 480 mg per day for 3 to 6 days cured 9 of 9 such infections. WR 171669 administered at 1.0 gm per day for 3 days cured 6 of 6 volunteers infected with the Smith strain. Large doses of both drugs evoked gastrointestinal symptoms - nausea, vomiting, abdominal pain and diarrhea.

Based upon additional data (3) it was believed that the biquinate salt of halofantine would enhance the bioavailability of halofantrine. Both compounds were assessed in parallel studies against infections of the Vietnam Smith strain and also included was a re-assessment of WR 122455.

A. WR 171669AM (BN: BK 64002)

The activity of the chloride salt of halofantrine is detailed in Table 4, and summarized in Tables 5 and 6. The data indicate that doses of 2.92, 5.83, or 11.67 mg base per kg (x 3 days) had either no effect or a suppressive effect upon primary Vietnam Smith parasitemias. Retreatment with a dose of 5.83 mg base per kg (x 3 days) in two monkeys only suppressed parasitemia. In four Aotus retreated with a dose of 11.67 mg base per kg (x 3 days), the parasitemia was suppressed in two monkeys, and cleared in two; the infection was cleared in one of the latter animals.

A dose of 23.3 mg base per kg (x 3 days) only suppressed parasitemia in two monkeys and cleared the parasites, but did not cure, in two Aotus. Parasitemias were cleared in 4 of 4 Aotus with a dose of 46.6 mg base per kg (x 3 days), but infections were not cured. One of two recrudescences was cured with a dose of 93.2 mg base per kg (x 3 days). No apparent toxic reactions occurred.

Four treatment failures were included in the WR 122455 assessment.

B. WR 171669AP (BN: BL 08009)

The biquinate salt of halofantrine was evaluated at the same doses as WR 171669AM. Detailed results are presented in Table 7, and summarized in Tables 8 and 9. Primary parasitemias were either not affected or suppressed with doses of 2.92, 5.83 or 11.67 mg base per kg (x 3 days). Retreatment with a dose of 5.83 mg base per kg (x 3 days) suppressed parasitemia, and retreatment with a dose of 11.67 mg base per kg (x 3 days) suppressed parasitemia in one Aotus and cleared parasitemia in 3 of 3 monkeys. Infections in the latter three monkeys were not cured.

Parasitemias in 6 of 6 Aotus retreated with a dose 23.3 mg base per kg (x 3 days) were cleared, but infections were not cured. Retreatment with a dose of 46.6 mg base per kg (x 3 days) cured the infection in 1 of 3 Aotus, and cleared parasitemias only in 2 of 3 monkeys.

Recrudescences in five Aotus were treated with WR 122455.

C. WR 122455AF (BN: AX26839)

Assessment of the hydrochloride salt of WR 122455 included primary Vietnam Smith parasitemias and recrudescences following multiple re-treatments with either WR 171669AM or WR 171669AP. The detailed activity is presented in Table 10 and summarized in Tables 11 and 12.

A dose of 2.92 mg base per kg (x 3 days) suppressed parasitemia in 2 of 2 Aotus. Two of 2 primary parasitemias were cleared at a dose of 5.83 mg base per kg (x 3 days), and the infection cured in one monkey. A dose of 11.67 mg base per kg (x 3 days) cured 2 of 2 primary infections.

Recrudescent parasitemias after re-treatments with either WR 171669AM or WR 171669AP, were cleared with WR 122455 at a dose of 2.92 mg base per kg (x 3 days); the infection in one Aotus was cured. Treatment with WR 122455 at a dose of 5.83 or 11.67 mg base per kg (x 3 days) respectively cured 7 of 7 and 4 of 4_{x} treatment failures with either WR 171669AM or WR 171669AP.

CONCLUSION

At the doses used, halofantrine (WR 171699AM) was essentially inactive against infections in Aotus of the multi-drug resistant Vietnam Smith strain of P. falciparum. In a total of 22 treatments, parasitemia was cleared in 9 cases with cure of only two infections. The biquinate salt (WR 171699AP) of halofantrine cleared 10 parasitemias in a total of 21 treatments, but only one infection was cured. The effective antimalarial activity of WR 122455 was evidenced by the parasite clearance in 17 of 19 treatments, and infection cure in 15 of 19 treatments. All treatment failures with either WR 171669AM or WR 171669AP were cured by WR 122455.

TABLE 4

DETAILED ACTIVITY OF WR 171669AM (BK 64002) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
11518	2.92	3	18	17	149	81	462	426	Re-Rx, higher dose			
11873	2.92	2	47	13	107	122	195	169	Re-Rx, higher dose			
11517	5.83	4	13	13	211	90	249	373	Re-Rx, higher dose			
11702	5.83	4	368	43	382	373	1064	766	Re-Rx, higher dose			
11518r	5.83	426	409	382	186	15	106	63	159	Re-Rx, higher dose		
11873r	5.83	169	38	364	86	53	10	60	85	Re-Rx, higher dose		
11740	11.67	4	27	39	391	214	461	995	Re-Rx, higher dose			
11904	11.67	3	20	18	51	24	212	82	Re-Rx, higher dose			
11517r	11.67	373	218	419	72	0.7	0.4	<0.01	<0.01	<0.01	>0.01	Re-Rx
11702r	11.67	766	595	1136	792	141	142	164	331	59	Re-Rx, higher dose	
11518rr	11.67	159	58	7	0.3	0.4	<0.01	0	0	0	0	0
11873rr	11.67	85	115	31	25	1	1	<0.01	<0.01	0	<0.01	0
11740r	23.3	995	852	995	427	497	73	26	0.8	0.2	Re-Rx, Higher dose	
11904r	23.3	82	80	71	2	0.7	0.2	<0.01	<0.01	0	<0.01	0
11517rr	23.3	> 0.01	0.4	1	<0.01	0	0	0	0	0	0	0
11702rr	23.3	59	146	1	2	0.3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
11740rr	46.6	0.2	0.3	<0.01	<0.01	<0.01	0	0	0	0	0	0
11904rr	46.6	0.2	1	0.2	<0.01	0	0	0	0	0	0	0
11517rrr	46.6	<0.01	<0.01	<0.01	<0.01	0	0	0	0	<0.01	<0.01	<0.01
11702rrr	46.6	<0.01	<0.01	<0.01	0	0	0	0	0	0	0	<0.01
11740rrr	93.2	37	151	115	3	0.2	<0.01	0	<0.01	0	0	0
11702rrrr	93.2	0.4	1	3	2	0.6	0.4	0.3	1	0.7	4	0.7

TABLE 5

SUMMARY OF THE ACTIVITY OF WR 171669AM (BK 64002) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Px to Parasite Clearance	Days from Final Rx To Recrudescence	Notes
		None	Suppressed	Cleared			
11518	2.92	+			n.a.	n.a.	Re-Rx, higher dose
11873	2.92		±		n.a.	n.a.	Re-Rx, higher dose
11517	5.83		±		n.a.	n.a.	Re-Rx, higher dose
11702	5.83	+			n.a.	n.a.	Re-Rx, higher dose
11518r	5.83		±		n.a.	n.a.	Re-Rx, higher dose
11873r	5.83		+		n.a.	n.a.	Re-Rx, higher dose
11740	11.67	+			n.a.	n.a.	Re-Rx, higher dose
11904	11.67		+		n.a.	n.a.	Re-Rx, higher dose
11517r	11.67		+		n.a.	n.a.	Re-Rx, higher dose
11702r	11.67		±		n.a.	n.a.	Re-Rx, higher dose
11518rr	11.67			+	5	n.a.	Cured
11873rr	11.67			+	10	36	Rx, new drug
11740r	23.3		+		n.a.	n.a.	Re-Rx, higher dose
11904r	23.3			+	10	29	Re-Rx, higher dose
11517rr	23.3			+	4	21	Re-Rx, higher dose
11702rr	23.3		+		n.a.	n.a.	Re-Rx, higher dose

TABLE 5 (CONT'D)

SUMMARY OF THE ACTIVITY OF WR 171669AM (BK 64002) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Px to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
11740rr	46.6			+	5	20	Re-Rx, higher dose
11904rr	46.6			+	4	8	Rx, new drug
11517rrr	46.6			+	4	5	Rx, new drug
11702rrr	46.6			+	3	6	Re-Rx, higher dose
11740 rrr	93.2			+	8	n.a.	Cured
11702rrrr	93.2		+		n.a.	n.a.	Rx, new drug

TABLE 6

SUMMARY OF THE ANTIMALARIAL ACTIVITY OF WR 171669AM (BK 64002) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

MALARIA	DOSE mg/kg		PRIMARY TREATMENTS		REPEAT TREATMENTS		TOTAL TREATMENTS	
STRAIN	TOTAL	DAILY	CLEARED	CURED	CLEARED	CURED	CLEARED	CURED
Smith	8.76	2.92	0/2	0/2			0/2	0/2
	17.49	5.83	0/2	0/2	0/2	0/2	0/4	0/4
	35.01	11.67	0/2	0/2	2/4	1/4	2/6	1/6
	69.9	23.3			2/4	0/4	2/4	0/4
	139.8	46.6			4/4	0/4	4/4	0/4
	279.6	93.2			1/2	1/2	1/2	1/2

TABLE 7

DETAILED ACTIVITY OF WR 171669AP (BL 08009) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
11780	2.92	3	18	30	293	87	160	173	Re-Rx, higher dose			
11811	2.92	2	49	35	204	151	315	461	Re-Rx, higher dose			
11781	5.83	3	8	15	102	42	80	320	Re-Rx, higher dose			
11899	5.83	2	18	28	83	71	200	45	Re-Rx, higher dose			
11780r	5.83	173	240	337	22	2	0.4	<0.01	<0.01	<0.01	<0.01	Re-Rx
11811r	5.83	461	284	87	17	1	0.2	<0.01	<0.01	<0.01	<0.01	Re-Rx
11779	11.67	4	19	20	42	64	204	102	Re-Rx, higher dose			
11898	11.67	2	16	15	5	3	17	4	Re-Rx, higher dose			
11781r	11.67	320	256	58	1	0.6	<0.01	<0.01	<0.01	0	0	<0.01
11899r	11.67	45	209	84	3	2	0.4	<0.01	<0.01	<0.01	0	0
11780rr	11.67	<0.01	<0.01	<0.01	<0.01	0	0	0	0	0	0	0
11811rr	11.67	<0.01	0.4	<0.01	<0.01	0	0	0	0	0	0	0
11779r	23.3	102	140	105	43	0.8	0.7	<0.01	<0.01	0	0	0
11898r	23.3	4	48	20	0.5	0.07	0.3	<0.01	<0.01	0	0	0
11781rr	23.3	<0.01	<0.01	<0.01	<0.01	0	0	0	0	0	0	0
11899rr	23.3	<0.01	0	0	0	0	0	0	0	0	0	0
11780rrr	23.3	17	13	10	0.4	<0.01	0	0	0	0	0	0
11811rrr	23.3	0.3	1	0.9	<0.01	0	<0.01	0	0	0	0	0
11779rr	46.6	<0.01	<0.01	<0.01	<0.01	0	0	<0.01	0	0	0	0
11898rr	46.6	<0.01	<0.01	<0.01	<0.01	0	0	0	0	0	0	0
11781rrr	46.6	0.6	1	0.4	<0.01	0	0	0	0	0	0	0

TABLE 8

SUMMARY OF THE ACTIVITY OF WR 171669AP (BL 08009) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Px to Parasite Clearance	Days from Final Px To Recrudescence	Notes
		None	Suppressed	Cleared			
11780	2.92		±		n.a.	n.a.	Re-Rx, higher dose
11811	2.92	+			n.a.	n.a.	Re-Rx, higher dose
11781	5.83		±		n.a.	n.a.	Re-Rx, higher dose
11899	5.83		±		n.a.	n.a.	Re-Rx, higher dose
11780r	5.83		±		n.a.	n.a.	Re-Rx, higher dose
11811r	5.83		+		n.a.	n.a.	Re-Rx, higher dose
11779	11.67		+		n.a.	n.a.	Re-Rx, higher dose
11898	11.67		+		n.a.	n.a.	Re-Rx, higher dose
11781r	11.67		+		n.a.	n.a.	Re-Rx, Higher dose
11899r	11.67			+	9	13	Re-Rx, higher dose
11780rr	11.67			+	4	20	Re-Rx, higher dose
11811rr	11.67			+	4	25	Re-Rx, higher dose
11779r	23.3			+	8	11	Re-Rx, higher dose
11898r	23.3			+	8	11	Re-Rx, higher dose
11781rr	23.3			+	4	21	Re-Rx, higher dose
11899rr	23.3			+	2	27	Rx, new drug
11780rrr	23.3			+	5	31	Rx, new drug
11811rrr	23.3			+	6	20	Rx, new drug
11779rr	46.6			+	7	25	Rx, new drug
11898rr	46.6			+	4	24	Rx, new drug
11781rrr	46.6			+	4	n.a.	Cured

TABLE 9

SUMMARY OF THE ANTIMALARIAL ACTIVITY OF WR 171669AP (BL 08009) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

MALARIA	DOSE mg/kg		PRIMARY TREATMENTS		REPEAT TREATMENTS		TOTAL TREATMENTS	
STRAIN	TOTAL	DAILY	CLEARED	CURED	CLEARED	CURED	CLEARED	CURED
Smith	8.76	2.92	0/2	0/2			0/2	0/2
	17.49	5.83	0/2	0/2	0/2	0/2	0/4	0/4
	35.01	11.67	0/2	0/2	1/4	0/4	1/6	0/6
	69.9	23.3			6/6	0/6	6/6	0/6
	139.8	46.6			3/3	1/3	3/3	1/3

TABLE 10

DETAILED ACTIVITY OF WR 122455AF (AX 26839) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
11492	2.92	4	11	20	80	42	25	7	2	0.4	1	0.3
11876	2.92	3	58	40	231	130	10	3	1	0.2	0.5	0.1
11517rrrr	2.92	0.1	0.4	0.2	<0.01	<0.01	0	0	0	0	0	0
11780rrrr	2.92	<0.01	0	0	0	0	0	0	0	0	0	0
11544	5.83	3	10	20	16	9	0.7	0.2	<0.01	0	0	0
11875	5.83	6	69	51	101	22	0.6	0.3	0	0	0	0
11492r	5.83	2	1	0.2	0.2	<0.01	<0.01	0	0	0	0	0
11876r	5.83	<0.01	<0.01	<0.01	0	0	0	0	0	0	0	0
11779rrr	5.83	60	82	14	1	0.2	0.1	0.3	<0.01	<0.01	0	0
11898rrr	5.83	10	13	1	0.4	0	0	0	0	0	0	0
11904rrr	5.83	5	2	2	0.2	<0.01	0	0	0	0	0	0
11811rrrr	5.83	0.3	1	1	0.8	<0.01	0	0	0	0	0	0
11780rrrrr	5.83	2	1	0.7	<0.01	0	0	0	0	0	0	0
11773	11.67	4	55	64	10	1	0.1	<0.01	0	0	0	0
11775	11.67	3	42	19	15	2	0.1	<0.01	0	0	0	0
11544r	11.67	0.1	0.5	0.4	0.2	<0.01	0	0	0	0	0	0
11873rrr	11.67	0.1	<0.01	<0.01	<0.01	0	0	0	0	0	0	0
11899rrr	11.67	2	11	0.5	0.4	<0.01	0	0	0	0	0	0
11702rrrrr	11.67	0.1	0.3	0.5	<0.01	0	0	0	0	0	0	0

TABLE 11

SUMMARY OF THE ACTIVITY OF WR 122455AF (AX 26839) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Px to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
11492	2.92		+		n.a.	n.a.	Re-Rx, higher dose
11876	2.92		+		n.a.	n.a.	Re-Rx, higher dose
11517rrrr	2.92			+	5	n.a.	Cured
11780rrrr	2.92			+	1	46	Re-Rx, higher dose
11544	5.83			+	8	40	Re-Rx, higher dose
11875	5.83			+	7	n.a.	Cured
11492r	5.83			+	6	n.a.	Cured
11876r	5.83			+	4	n.a.	Cured
11779rrr	5.83			+	9	n.a.	Cured
11898rrr	5.83			+	4	n.a.	Cured
11904rrr	5.83			+	5	n.a.	Cured
11811rrrr	5.83			+	5	n.a.	Cured
11780rrrrr	5.83			+	4	n.a.	Cured
11773	11.67			+	7	n.a.	Cured
11775	11.67			+	7	n.a.	Cured
11544r	11.67			+	5	n.a.	Cured
11873rrr	11.67			+	4	n.a.	Cured
11899rrr	11.67			+	5	n.a.	Cured
11702rrrrr	11.67			+	4	n.a.	Cured

TABLE 12

SUMMARY OF THE ANTIMALARIAL ACTIVITY OF WR 122455AF (AX 26839) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

MALARIA	DOSE mg/kg		PRIMARY TREATMENTS		REPEAT TREATMENTS		TOTAL TREATMENTS	
STRAIN	TOTAL	DAILY	CLEARED	CURED	CLEARED	CURED	CLEARED	CURED
Smith	8.76	2.92	0/2	0/2	2/2	1/2	2/4	1/4
	17.49	5.83	2/2	1/2	7/7	7/7	9/9	8/9
	35.01	11.67	2/2	2/2	4/4	4/4	6/6	6/6

ASSESSMENT OF THE ANTIMALARIAL ACTIVITIES OF 2-FLUORO-L-HISTIDINE AND 2-IODO-HISTIDINE AGAINST INFECTIONS OF THE UGANDA PALO ALTO STRAIN OF PLASMODIUM FALCIPARUM

A pilot evaluation (6) of 2-fluoro-l-histidine (WR 251853AA; BN: BK 70877) indicated that a total daily dose of 25.0 mg base per kg (x 7 days) suppressed parasitemia of the Uganda Palo Alto strain in 1 of 2 treated Aotus. Parasitemias in 2 of 2 Aotus were suppressed by a total daily dose of 50.0 mg base per kg (x 6 days); however, both animals died, on the sixth day of treatment, of probable drug toxicity. Since in vitro studies at the Malaria Section Laboratory, National Institutes of Health, Bethesda, Md., have shown that 2-fluoro-l-histidine will inhibit both parasite growth and knob formation of parasitized erythrocytes, additional in-vivo studies were considered to be of value.

Accordingly, Dr. Lindsey Panton (from NIH) was at Gorgas Memorial Laboratory for one month in a cooperative effort to re-evaluate 2-fluoro-l-histidine and a less toxic histidine, 2-iodo-histidine. Drugs were provided by Dr. Panton. Also, Dr. Carter Atkinson, Case Western Reserve University (Dr. Aikawas' laboratory), Cleveland, Ohio obtained tissue samples from treated and control monkeys for election microscopy determination of parasite sequestration.

A. 2-fluoro-l-histidine

This histidine was re-evaluated in a total of four Aotus infected with the Uganda Palo Alto strain (Tables 13 and 14). Two monkeys each were treated (intravenously) with a single 50.0 mg base per kg dose and each of two Aotus were treated (intravenously) with a single 100.0 mg base per kg dose. The 50.0 mg base per kg dose had no effect upon parasitemia in either monkey and one monkey was sacrificed for tissues on day 4, post treatment.

Parasitemia in Aotus 11379 was suppressed by a 100.0 mg base per kg dose. The parasite clearance (23 days post treatment) was not considered to be the result of drug action, but represented the length of a normal patent period (24.1 + 4.8 days) during the primary attack of infections with the Uganda Palo Alto strain of P. falciparum (7). The parasitemia in Aotus 11997 was not suppressed by a single 100.0 mg base per kg dose; the monkey was sacrificed on day 7 post treatment for tissues.

B. 2-iodo-histidine

This analogue of the amino acid, histidine, was first evaluated by a single dose administered intravenously to monkeys infected with the Uganda Palo Alto strain. The data presented in Tables 15 and 17 show that a dose of 200.0 or 400.0 mg per kg had no effect upon parasitemia in each of four Aotus.

Monkeys were then retreated each with a 100.0 mg per kg dose (oral) as follows: day 1 - one dose in the afternoon, day 2 - one dose in the morning and one in the afternoon. Results shown in Tables 16 and 18 that this course of treatment had no effect upon parasitemia and the animals succumbed to malaria infection.

CONCLUSION

The significant in vitro activity of these histidines was not evident in the in vivo evaluation, as measured by the lack of effect upon parasitemia. The examination of tissues by election microscopy is in progress.

TABLE 13

DETAILED ACTIVITY OF 2-FLUORO-L-HISTIDINE AGAINST INFECTIONS OF THE UGANDA PALO ALTO STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose* Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Rx	Day Post Treatment								
			1	1	2	3	4	5	6	7	8	9
11353	50.0 AM	10	100	60	408	311	634	521	338	580	284	265
	PM		151	310	80	409	506			471	426	382
11613	50.0 AM	6	231	109	992	701	1460					
	PM		423	487	350	1232	Sacrificed					
11379	100.0 AM	2	31	4	7	4	47	142	48	267	249	110
	PM		16	5	4	30	36			169	401	240
11997	100.0 AM	5	141	77	409	149	1172	595	1331	1510		
	PM		231	140	321	214	1170			Sacrificed		

*Intravenous administration

TABLE 14

SUMMARY OF THE ACTIVITY OF 2-FLUORO-L-HISTIDINE AGAINST INFECTIONS OF THE UGANDA PALO ALTO STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 1 * Mg/Kg	Response of Parasitemia to Rx			Days from Initial Px to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
11353	50.0	+			17		
11613	50.0	+			n.a.	n.a.	Sacrificed Day 4 post-Rx
11379	100.0		+		23		
11997	100.0	+			n.a.	n.a.	Sacrificed Day 7 post-Rx

* Intravenous administration

TABLE 15

DETAILED ACTIVITY OF 2-IODO-HISTIDINE AGAINST INFECTIONS OF THE UGANDA PALO ALTO STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg		Parasitemia per cmm x 10^3									
		Day Pre-Rx	Rx	Day Post Treatment								
			1	1	2	3	4	5	6	7	8	9
11486	200.0 AM	3	106	80	320							
	PM		118	142	400	Re-Rx, different dose and route						
11581	200.0 AM	2	40	25	284							
	PM		35	175	52	Re-Rx, different dose and route						
11585	400.0 AM	4	122	31	746							
	PM		144	609	290	Re-Rx, different dose and route						
12253	400.0 AM	2	134	71	639							
	PM		180	329	568	Re-Rx, different dose and route						

* Intravenous administration

TABLE 16

DETAILED ACTIVITY OF RETREATMENT WITH 2-IODO-HISTIDINE AGAINST INFECTIONS OF THE UGANDA PALO ALTO STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose* Mg/Kg	Parasitemia per cmm x 10^3									
		Day Pre-Rx	Day of Rx		Day Post Treatment						
			1	2	1	2	3	4	5	6	7	8
11486r	100.0 AM			187	568	846	781	817				
	PM	400		639	768			Died - malaria				
11581	100.0 AM			639	249	675	533	142	639			
	PM	52		710	568				Died - malaria			
11585r	100.0 AM			923	750	604	462	462	543			
	PM	290		959	320				Died - malaria			
12253r	100.0 AM			817	521	Died - malaria						
	PM	568		1101	604							

* Oral

TABLE 17

SUMMARY OF THE ACTIVITY OF 2-IODO-HISTIDINE AGAINST INFECTIONS OF THE UGANDA PALO ALTO STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x1 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Px to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
11486	200.0	+			n.a.	n.a.	Re.Rx, different dose and route
11581	200.0	+			n.a.	n.a.	Re-Rx, different dose and route
11585	400.0	+			n.a.	n.a.	Re-Rx, different dose and route
12253	400.0	+			n.a.	n.a.	Re-Rx, different dose and route

* Intravenous

TABLE 18

SUMMARY OF THE ACTIVITY OF 2-IODO-HISTIDINE AGAINST INFECTIONS OF THE UGANDA PALO ALTO STRAIN OF *PLASMODIUM FALCIPARUM*

Monkey No.	Daily * Dose Mg/Kg	Response of Parasitemia to Rx			Days from Initial Px to Parasite Clearance	Days from Final Rx To Recrudescence	Notes
		None	Suppressed	Cleared			
11486r	100.0	+			n.a.	n.a.	Died Day 5, post Rx, malaria
11581r	100.0	+			n.a.	n.a.	Died Day 5, post Rx, malaria
11585r	100.0	+			n.a.	n.a.	Died Day 5, post Rx, malaria
12253r	100.0	+			n.a.	n.a.	Died Day 2, post Rx, malaria

* Administered orally - Day 1 - P.M., Day 2 - A.M.& P.M.

PILOT EVALUATION OF SODIUM ARTESUNATE AGAINST INFECTION OF THE UGANDA PALO ALTO STRAIN OF PLASMODIUM FALCIPARUM

Qinghaosu (QHS), artemisinin) was isolated by Chinese chemists in 1971 from the herb Artemisia annua (8). The herb had been used for more than 10 centuries in China as treatment for fevers and malaria. QHS has been used successfully against P. falciparum infections in man either chloroquine-sensitive or chloroquine - resistant strains. A derivative of QHS, is the water soluble sodium artesunate, more active than QHS against chloroquine - resistant and chloroquine - sensitive strains of P. berghei. Sodium artesunate has proven to be effective in human cases of cerebral malaria. There have been no systematic studies evaluating sodium artesunate in the P. falciparum - Aotus model. The present evaluation was limited to but a single Aotus because of the restricted availability of the drug.

Doses of sodium artesunate, each at 30.0 mg per kg, were administered intravenously on days 1 and 2 of treatment, to Aotus 11488 (Tables 19 and 20). The parasitemia was suppressed during days 2 through 6 post treatment, but then increased to pre-treatment level. The parasitemia cleared on day 18 after the first day of treatment.

It is to be noted that the drug was not completely water soluble.

CONCLUSION

Based upon one Aotus infected with the chloroquine-sensitive Uganda Palo Alto strain, a 30.0 mg per kg dose (x 2 days) suppressed the parasitemia. Further studies are pending upon the availability of additional drug.

TABLE 19

DETAILED ACTIVITY OF Na ARTESUNATE AGAINST INFECTIONS OF THE UGANDA PALO ALTO STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose * Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Rx		Day Post Treatment							
			1	2	1	2	3	4	5	6	7	8
11488	30.0 A.M.	251	160	57	67	28	10	4	36	7	231	150
	P.M.		247	109	37			1	43	66	150	378

* Intravenous

TABLE 20

SUMMARY OF THE ACTIVITY OF Na ARTESUNATE AGAINST INFECTIONS OF THE UGANDA PALO ALTO STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 2 * Mg/Kg	Response of Parasitemia to Rx			Days from Initial Px to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
11488	30.0		±		18	n.a.	Cured

* Intravenous

ADAPTATION TRIALS IN AOTUS OF THE UNC-W2-MEF CLONE OF PLASMODIUM FALCIPARUM

Although not a primary goal, it was stipulated at the inception of this contract 10 years ago that new strains of P. falciparum or P. vivax may have to be adapted to Aotus to accomplish a specific purpose. The CDC Indochina III strain of P. falciparum is resistant to Fansidar and chloroquine. The UNC-W2 clone of this strain was made resistant to mefloquine during two years of continuous mefloquine pressure in vitro (3). The UNC-W2-MEF clone is 4x more resistant to mefloquine than the parent clone.

A culture of the UNC-W2-MEF clone was provided by MAJ W.K. Milhous, Walter Reed Army Institute of Research, for adaptation trials to Aotus. The genealogy shows, to date, the adaptation trials in Aotus of diverse origin. Details are presented in Table 21. Two monkeys, 8348 and 11614, were each inoculated intravenously with approximately 100 x 10^6 parasites from the in vitro culture.

Blood films obtained from Colombian Aotus 8348 (splenectomized) were negative for 14 consecutive days and the monkey was reinoculated with a frozen sample. A patent infection was established (Table 21) and parasites subinoculated to other recipients.

A patent parasitemia was not established during a 44 day examination period in Panamanian Aotus 11614 (splenectomized) following inoculation of parasites obtained from culture. Infected blood from 8348 was inoculated into 11614, but only a low-grade parasitemia (parasites detectable in thick blood films) ensued for 31 days. A second subinoculation of infected blood from 8348 did produce a countable parasitemia - maximum of 540 per mm^3.

Parasite subinoculation from 8348 to Panamanian Aotus 11742 (splenectomized) produced patent period of 12 days, with a maximum parasitemia of 450 per cmm.

A hybrid Aotus (Colombian x Panamanian), not surgically altered, was inoculated with parasites from 8348. To date, a patent infection has not been established.

CONCLUSION

To date, the highest parasitemias obtained during this monkey adaptation procedure of the UNC-W2-MEF clone has been in a splenectomized Aotus of Colombian origin. These parasitemias, however, are considerably lower than usually seen for well-adapted P. falciparum strains in Aotus. Moreover, high parasitemias have not yet occurred in Panamanian Aotus. Adaptation trials of this clone will continue.

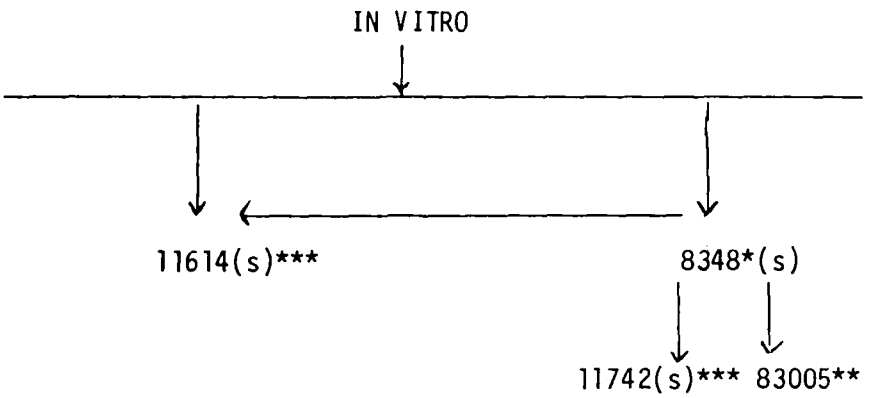
TABLE 21

DETAILS OF ADAPTATION TRIALS IN AOTUS OF THE UNC-W2-MEF CLONE OF PLASMODIUM FALCIPARUM

Monk No.	No. Parasites Inoc. x 10^6	Pre-Patent PD.-Days	Maximum Parasitemia Per mm^3	Patent Day	Patent PD-Days	Notes
8348(S,C)	100	--	--	--	--	Neg.14 Days-Reinoc with frozen sample
8348r	13	19	28,000 60,000 20,000	30 60 90	113	Subinoc 11614-Days 12 & 58 Subinoc 11742-Day 90 Subinoc 83005-Day 97
11614(S,P)	100	--	--	---	--	Neg. 44 Days
11614r	9	1	<10	--	31	
11614rr	44	1	540	10	38	
11742(S,P)	60	1	450	5	12	
83005(H)	2					Neg. 19 Days

S = Splenectomized
C= Colombian Aotus
P= Panamanian Aotus
r= Re-inoculated
H= Hybrid (Panamanian M, Colombian F) Aotus

GENEALOGY OF ADAPTATION TRIALS IN AOTUS OF THE UNC-W2-MEF CLONE OF PLASMODIUM FALCIPARUM



(s)= Splenectomized
* = Aotus of Colombian origin
** = Hybrid (Panamanian M, Colombian F)
*** = Aotus of Panamanian origin

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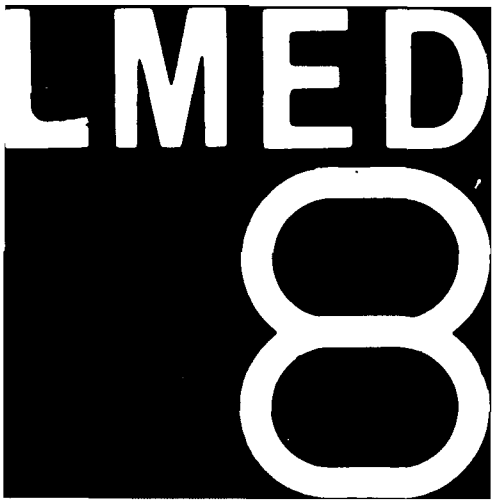
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